Structure-based development of target-specific compound libraries

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The success or failure of a small-molecule drug discovery project ultimately lies in the choice of the scaffolds to be screened – chosen from among the many millions of available compounds. Therefore, the methods used to design compound screening libraries are key for the development of new drugs that target a wide range of diseases. Currently, there is a trend towards the construction of receptor-structure-based focused libraries. Recent advances in high-throughput computational docking, NMR and crystallography have facilitated the development of these libraries. A structure-based target-specific library can save time and money by reducing the number of compounds to be experimentally tested, also improving the drug discovery success rate by identifying more-potent and specific binders.

The main goal when designing screening libraries is to limit the number of compounds requiring experimental testing while maximizing the hit rate. Currently, drug-like compounds that are stored in public and private compound libraries do not yet represent the full range of chemical space, which can be exploited to develop new therapeutics. In fact, it has been proposed that, given a lead fragment-scaffold, an infinite number of variations are theoretically possible [1], whereas others speculate that there are 10^{10}–10^{50} possible compounds available for drug design, reviewed in Ref. [2]. A mathematical framework for studying the number of molecules required to sample, adequately, a given property space has also been published [3]. Whatever the actual number is, the generation of focused target-specific libraries is advantageous, especially after the partial failure of diversity in combinatorial libraries in the past [4]. A comprehensive survey on combinatorial libraries is published annually [5].

Experimental or computer-derived structural data relating to a drug target provide precious information, focusing the chemical space of compound libraries according to restraints dictated by the ligand–receptor interaction energy. Moreover, focused libraries not only reduce waste by eliminating *a priori* compounds that are unlikely to bind to the target (thus saving time and money), but could also eventually lead to an increase in the potency or specificity of binders, as it has been shown with inhibitors for c-Src kinase [6]. Designing *de novo* compound libraries based on drug-target-specific protein structures also enables the discovery of compounds that could be missed by conventional library generation and screening techniques. This theory is backed up by several reports from the pharmaceutical industry that indicate that, although diversity is important for library design, an active compound can be hidden by the vast 'noise' of the database in which it is contained [7,8]. Moreover, when compared with ligand-based design of chemical libraries, the structure-based approach does not need a collection of pre-existing ligands, nor is it biased towards known scaffolds. Structure-based library design is a particularly useful tool, either in situations where compound diversity is sought or for guiding combinatorial chemistry in the stages of lead optimization. In the latter case, additional drug-like filters will certainly improve the therapeutic profile of the drug candidates.

In the past, 3D structures (experimentally derived or homology models) have been used in the drug-design process, mainly to guide structure-based lead optimization for improving potency or selectivity (see Ref. [9] for a list of marketed drugs derived from these structure-based approaches). Later, experimental protein structures were used to identify (or for the *in silico* screening of) chemical libraries or fragments. The idea of bioevaluating a structure-based, *in-silico*-designed focused library provides an alternative to the traditional HTS methods of lead discovery [10,11]. With

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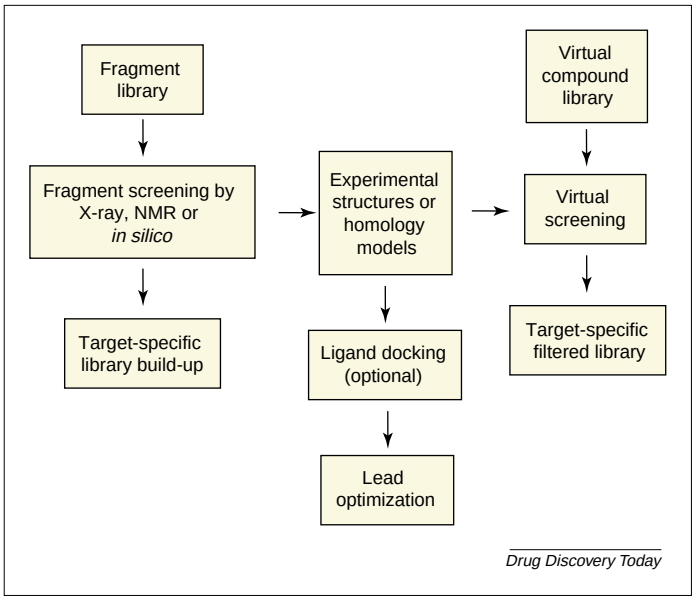

FIGURE 1

The structure-based library design process. Focused libraries derived from a fragment-based approach or through target-specific filters reduce not only the number of compounds to be bioevaluated, but also might result in leads with higher potency and selectivity. Experimental structures and models are used in the library-design process to guide lead optimization. Fragment screening facilitates the build-up of a library, whereas virtual screening can be applied as a compound filter.

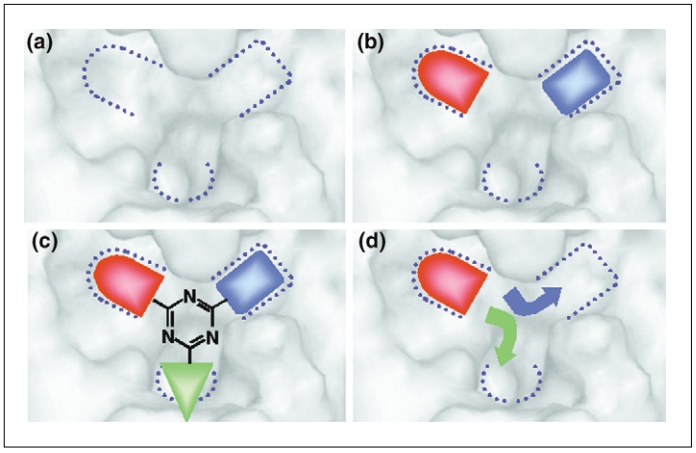

FIGURE 2

Fragment-based compound library generation. (a) A binding site consisting of three pockets. **(b)** Molecular fragments are identified as binding to pockets by experimental or *in silico* screening. **(c)** A compound library is generated by linking molecular fragments. **(d)** Another possibility: generating a compound library by 'growing out' from one fragment into neighboring pockets. Reproduced from Ref. [13].

the recent advent of high-throughput NMR [12] and crystallography [13], the use of these traditional techniques shifted (from tools for structure determination to tools for fragment screening), thus enabling the construction of targeted fragment-based chemical libraries. A schematic view of the structure-based library-design process is shown in Figure 1. An excellent review on the role of structural biology in drug discovery has recently been published [9].

As the benefits of building target-specific compound libraries are being realized, more groups are publishing successful drug-design efforts using this technique. In this review we discuss recent successes of the structure-based design of focused drug-target-specific compound libraries.

In-silico-based design of focused libraries

Structural knowledge obtained from ligand–protein complexes (determined either experimentally or *in silico*) can primarily aid the design of focused structure-based libraries by optimizing ligand–receptor complementary interactions, in an attempt to improve potency and/or specificity. The number of high-resolution structures stored in the Protein Databank [14] is rising exponentially year after year and each new structure reveals more details, aiding the prediction of drug–receptor interactions. This has also widened the possibility of building homology models for relevant therapeutic targets by expanding the availability of protein templates.

When a 3D representation of the receptor and a chemical library are available, virtual screening (based on ligand docking) followed by scoring provides a method that imposes a structure-based filter on chemical libraries by selecting a subset of compounds that show steric and electrostatic complementarity with the binding pocket. Current trends and developments in docking and virtual-screening procedures are described elsewhere [15–19]. Virtual screening has been applied in many drug discovery projects, the latest successes include: discovery of a novel type of inhibitor of peptide deformylase [20] and identification of novel epidermal growth factor receptor (EGFR) inhibitors with antiproliferative activity against cancer cells [21] (both using the ICM scoring function [22]); low micromolar inhibitors of the tyrosine kinase p56-Lck SH2 domain [23]; and the discovery, using DrugScore [24], of submicromolar ligands that inhibit the NK-1 G-protein-coupled receptor. Computational fragment-based *de novo* methods (see Ref. [25] for a review on this methodology and recent examples of *de novo* design) where docked fragments are scored and then linked (the 'dock and link' strategy) or grown toward the neighboring available space ('seed and grow'), see Figure 2, are particularly amenable to the knowledge-based design of combinatorial libraries. Although *de novo* methods can design novel compounds (not just those in a pre-existing chemical library), not all of them will be amenable to chemical synthesis. An excellent overview about the computer-aided structure-based drug discovery process has been published recently [26].

Computer-aided structure-based design relies on X-ray crystallography as the main source of structural data. Thus, it is always wise to be aware of the inherent limitations of deriving atomic models from electron-density data (see Ref. [27] for an interesting review on the application and limitations of X-ray data in structure-based drug design). When the receptor structure is derived from a homology model, further precaution should be exercised regarding the validity of the model (see Ref. [28] for a review on the use of models derived from homology in drug design). Of course, there are also inaccuracies in the docking and scoring algorithms, however, *in-silico*-based methods can be very valuable – as is evident in the following recently published examples.

Working on a homology model of the immune-modulating target Janus Kinase 3 (JAK3) protein tyrosine kinase, Adams *et al.* [29] docked the micromolar inhibitor oxindole, and key ligand–receptor interactions were identified, which were then used to guide the construction of a combinatorial library containing 700 compounds.

The library was constructed by substituting readily available aldehydes onto the 5-position of the oxindole ring. It was postulated that the aldehydes would interact with a hydrophobic pocket that had been predicted by modeling. The library was successfully designed to interact with the variable region into which the pyrrole ring of oxindole was projecting, leading to the identification of nanomolar leads.

Gerlach *et al.* [30] have recently undertaken an interesting study on library design, based on privileged structures. By applying docking and scoring of a privileged compound library [31] to thrombin and trypsin, top-ranking compounds were synthesized, co-crystalized and their binding affinity was determined. This structural and biochemical characterization will guide the design of structure-based combinatorial libraries and their compounds will be docked into putative binding sites in the search for leads with improved potency [30].

Docking, followed by detailed analysis of the ligand in the binding pocket and the subsequent design of a library based on this pose, can lead to the development of an enriched library of binders. Wu *et al.* [32] have used their in-house docking software (LIDAEUS) to dock compounds to the crystal structure of cyclin-dependent kinase-2 (CDK-2), and they used the best hits to generate a hypothesis on the docking pose and to produce more-potent compounds within the screening library. Inhibition of CDKs can have anticancer effects and, therefore, have been of great interest to computational chemists attempting to design target-specific libraries. It was found that an electron-donating substituent linked to the pyrimidyl amino group would improve interactions in the pocket and, hence, the library screened was based around these improvements. Aldose reductase (AR) inhibitors have also been identified using a similar combination of docking and library development. Using a series of knowledge-based filters and a detailed understanding of the atomic details of the residues contained within the AR binding site, Kraemer *et al.* [33] constructed a focused library of 1261 compounds, from an initial library of 260,000 compounds. From this focused library, six inhibitors were identified in the micromolar range.

A successful focused library has been constructed to identify inhibitors for the checkpoint kinase-1 receptor, which is a key enzyme for preventing DNA damage in the Gap2/mitosis (G2/M) checkpoint in the cell cycle [34]. Using target-specific knowledge-based filters, the library was designed to be favorable for the identification of ATP-competitive inhibitors. For example, all members of the library were capable of interacting with the peptidic nitrogen of Cys87 and the amide carbonyl of Glu85 to mimic the purine ring of ATP.

Using a combination of modeling and docking to design a library, Ranise *et al.* [35], were able to identify thiocarbamates (TCs) as a novel class of non-nucleoside reverse transcriptase inhibitors (NNRTIs), isosteres of phenethylthiazolylthiourea (PETT) derivatives. These inhibitors work by interacting with reverse transcriptase (RT), preventing viral RNA from converting to the viral DNA that infects healthy cells. NNRTIs bind to an allosteric pocket contained within the non-nucleoside binding site (NNBS), which is ~10Å away from the polymerase active site. The design of new NNRTIs is crucial because of the nonconserved amino acid sequence of NNBS, which favors the rapid selection of NNRTI-resistant viruses. The Dock 4.0 algorithm was used to develop hypothetical TC binding modes. These models were then used for the structure-based development of TC libraries. From these models it was noted that there was likely to be a polar interaction between one of the imidic oxygen atoms of TC and the Glu138 of the receptor, and this led to the incorporation of alcohol building blocks (into the library construction). Isothiocyanate building blocks were also included in the TC library synthesis, as a result of analyzing the binding pocket. This showed that binding affinity could be increased if hydrophobic contacts were made with Leu100, Lys101, Val106, His235, Pro236 and Tyr318. The screening of this library produced improved activity compared with previously generated NNRTIs, but there is still more optimization needed to make analogs with better resistance profiles.

X-ray crystal structure based design of compound libraries

For many years, crystallography has been considered to be a slow and expensive methodology, and its use in the drug-design process has been somewhat limited as a result. However, with the advent of high-throughput crystallography, the use of this technique as a screening tool is extending to fragment-based drug discovery [13,36]. It is very interesting that this advance was not driven by the pharmaceutical industry but, instead, by academic structural genomics initiatives [37].

The fragment-based methodology consists of the identification of smaller molecules of significantly lower molecular weight than normal drug molecules, binding to the target with lower affinity (usually in the millimolar range) than the standard hits. X-ray crystallography is an ideal tool in this task, used to assess atomic interactions with excellent accuracy. Once fragments binding to the target have been identified, they can be linked through a scaffold or one fragment can be taken as a starting point that grows into neighboring pockets (Figure 2). In either case a structure-based compound library is generated that can be bioassayed later. For a review on fragment-based techniques in drug discovery see Ref. [38]. An important limitation of a crystal structure approach to library design is the static nature of a crystal structure. A receptor–ligand structure solved by X-ray crystallography does not reflect the flexible nature of some ligand-binding pockets and therefore errors might arise in the library design. Fragment-based drug discovery techniques are sometimes limited by the impossible (or poor) chemistry required to link the fragments, this can result in a poorly optimized scaffold. However, the following examples highlight some of the recent successes of this method.

An example of a meticulous structure-based library design was reported recently by Kaila *et al.* [39]. They discovered quinic acid derivatives as sialyl Lewisx (sLex)-mimicking selectin inhibitors. Their paper reports an iterative approach to library building with each newly constructed library based on predicted interaction with the receptor. Leukocyte migration ensures the exposure of effector cells to their targets (e.g. inflammatory sites). The migration into target tissues is mediated by P- and E-selectin cell adhesion molecules, expressed on the vascular endothelium with tetrasaccharide sLex-containing ligands on the leukocyte cell surface. Cardiovascular disease, allergy and conditions such as stroke are some of the disease indications caused by excessive recruitment of leukocytes; therefore, understanding the interaction between sLex and selectins (and developing inhibitors of this interaction) could result in new

therapeutics. The co-crystal of E-selectin quinic acid was used to generate a model of P-selectin, facilitating the construction of eight virtual compound libraries (libraries A–H) based on quinic acid. Libraries A and B investigated the addition of aryl groups to the lead compound, which had the quinic acid scaffold but with modifications to the 1-hydroxy group. It was hoped these modifications would increase interaction with the protein surface. Some hits were identified for library B in which the aryl group interacts with the protein surface residue Tyr48. This was also a factor in the design of library F, which also sought to improve interactions with Tyr94, as well as Tyr48. Library C was built to improve the protein interactions observed from the compounds in library B, libraries D and E were designed to include compounds that could orientate in the direction of a small hydrophobic tunnel on the surface of P-selectin and libraries G and H explored amide substitutions – several diverse reagents were chosen. Eleven compounds demonstrated good P-selectin inhibition in the three test assays performed.

Small-molecule inhibitors of tissue factor (TF) VIIa have the potential to inhibit the coagulation cascade, which is triggered by the binding of TF VIIa to a cell surface TF that, in the case of disease state or during injury, can lead to thrombus formation. Parlow *et al.* [40] have used the crystal structure of TF VIIa to design inhibitors that have improved inhibitor potency and selectivity by designing a library containing a series of novel, substituted benzene analogs. Starting with a pyrazinone that had been previously discovered [41,42] to be a potent inhibitor of TF VIIa, moiety alterations were made that were based on the crystal structure, thus improving inhibitor binding. The carboxylate group of a key aspartic acid (Asp189) can mediate an ion pair with an amidine moiety that improves specificity in the S2 pocket, and a substituted *m*-aminophenyl ring also binds in this position. The crystal structure prompted the realization that forming hydrogen bonds with Gly216 could improve potency. Screening the library identified a compound (substituted with a ketone at the 2-position of the pyrazinone ring) that formed a hydrogen bond with Gly126. This study resulted in a molecule that specifically bound to TF VIIa with a K_i of 16 nM.

Researchers at Astex have recently been successful in employing high-throughput X-ray-crystallography screening to identify low-affinity fragment hits for a range of enzyme targets [43–45]. Low-affinity fragments are detected by changes in electron density and then these fragments are iteratively optimized using many protein–ligand co-crystals. For example, a potent and selective series of p38α mitogen-activated protein (MAP) kinase inhibitors were initially identified and the activities of these compounds were improved by exploiting the flexibility of the conserved DFG-binding loop. This resulted in a potent and selective inhibitor with an IC_{50} of 65 nM. Another series of p38α MAP kinase inhibitors was discovered by building a library of compounds that bind in a unique pose that allows both rings of the indolyl moiety to reside within the hydrophobic specificity pocket [44].

In another study by Terasaka *et al.* [46], adenosine deaminase, an anti-inflammatory target, was studied and potent non-nucleoside inhibitors were identified by building a library of merged fragments and testing them. Crystal structures of the fragments revealed conformational changes within the active site that resulted in a complex linker and library-design strategy.

NMR-based approaches to library design

A target-specific library can be generated via a structure-based approach by the application of NMR techniques. In the past few years NMR has found a niche in the drug discovery and lead screening fields of structural biology [47–49]. For example, SAR generated by NMR have been used in the past for the identification of inhibitors for both stromelysin and the DNA-binding domain of the human papillomavirus E2 protein, an important enzyme for tissue repair and a protein required for viral replication, respectively [50,51]. 'SAR by NMR' requires a large amount of pure, labeled protein and a high-field NMR spectrometer, which is sometimes considered a limitation for this method. NMR also has a limited applicability when trying to identify strong binders that exhibit slow dissociation and exchange [12].

'SAR by NMR' and related techniques have been extended by incorporating dual-amino-acid-selective labeling and site-directed non-native amino acid replacement (SNAAR). Selective detection of NMR resonances for a specific amino acid residue is used, thereby reducing the spectral complexity, and can be applied for larger molecules and systems. Selective labeling can be incorporated into just the binding region of the receptor [52,53].

If the approximate structural nature of the binding pocket or groove is known defined residues can be used to monitor changes within the pocket and/or the position of the ligand upon binding. In the most common use of NMR, ^{15}N labeling is used; however, tryptophan labeling with either ^{19}F or ^{13}C has also been used in NMR experiments to monitor ligand–protein interactions [54,55]. A method called RAMPED-UP enables several proteins to be studied at the same time, as well as differential changes in spectra that are monitored upon ligand binding [56]. All these techniques have the capability to be applied to build target-specific compound libraries based upon the protein structure [57,58].

The advantage of NMR is that very weak binders (in the millimolar range of activity) can be detected, along with the position within the receptor where binding occurs. The binding of a weak binder can provide important information for the development of a target-specific library and, using fragment-assembly techniques, can rapidly identify new binders. These low-affinity scaffolds can then be used for the parallel synthesis of analogs to develop higher-affinity binders.

Recently, an inhibitor of the B-cell leukemia 2 (Bcl-2) family of proteins, which are central regulators of programmed cell death, has been identified [59]. The potent inhibitor, which targets a protein–protein interaction site, was discovered using a combination of NMR-based screening, parallel synthesis and structure-based drug design. A focused library was generated based on the architecture of the binding cleft, which contains two distinct but proximal subsites. The library contained compounds that could bridge the gap between subsites 1 and 2 and link two moieties (4-fluorobiphenyl-4-carboxylic acid and 5-,6-,7-,8-tetrahydronaphthalen-1-ol), identified by NMR to bind with millimolar affinity. The compounds were further improved by incorporating, into the library, analogs with polar substitutions and a lipophilic group, reducing binding to human serum albumin and also enabled access to a deeper groove in the protein structure.

The protein tyrosine phosphatase (PTP) family of proteins is known to be a group of signaling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic

cycle and oncogenic transformation. PTP-1B, in particular, has been shown to be a negative regulator of insulin. A big problem in the design of PTP-1B inhibitors arises when trying to ensure specificity and trying to prevent interaction with other structurally similar phosphatases such as T cell phosphatase. NMR identified an initial weak binder that was optimized by screening structure-based libraries (utilizing a structurally specific phophotyrosine binding site within PTP-1B) [60]. This compound was highly charged, unable to enter cells as a result and, therefore, did not exhibit any cellular activity.

Fragments identified as binders by NMR were, once again, used to build a library of compounds to design a small-molecule, low affinity (micromolar), selective, *in-vitro*-active inhibitor of PTP-1B [61]. Fragments were selected that bound to the catalytic site and a second phosphotyrosine binding site. Structure-based assembly was then used to join these fragments to produce a library of potential binders that were subsequently screened. The authors note that this approach is advantageous because it starts from screening a small number of compounds with predefined properties and then allows for an expansion of the library to generate molecules with optimized affinity, selectivity and cellular activity.

Oxidoreductase enzymes have a conserved Rossmann fold, which enables the NAD(P)(H) co-factor to bind, and a unique domain that facilitates substrate specificity. In 2004, Sem *et al.* [62] combined computational techniques and NMR in the design of bidentate-ligand inhibitors of oxidoreductase. Structural subfamilies (named pharmacofamilies) were used to describe the key binding-site features, such as hydrogen bond donors and acceptors. Using computational methods, an initial compound was identified that binds in the nicotinamide site, this compound is referred to as a common ligand mimic (CLM), because it can be used, in principle, for other proteins from the same pharmacofamily. The orientation and position of the CLM is then determined using an experimental method called NMR SOLVE [63], which compares a reference ligand with the CLM. NMR SOLVE provides library expansion information, such as the position a linker should be placed to build the CLM into the specificity pocket. A focused library of 300 compounds was built by adding diverse elements to the linker. The initial CLM bound weakly in the micromolar range but the development of the focused library resulted in the identification of compounds with nanomolar activity.

Human factor Xa inhibitors have also been identified by NMR. A small, focused library was built that contained fragments of known active molecules that were chosen by referring to the structural motifs present in the receptor. The library was then enriched further, based on the NMR screening data [64]. The authors of this work have also evaluated the practicalities of applying this method to bovine serum albumin as a model for protein–ligand interactions [65].

Conclusions and outlook

Structural knowledge pertaining to a drug target could help to reduce the size of screening libraries or to design novel, focused libraries. Focused libraries can save time and money in the drug-design process by eliminating *a priori* compounds that are unlikely to bind to the target, also improving the drug-design success rate by identifying more-potent and specific binders. The general trend (from the late 1990s to the present day) is that libraries have become more target-focused and less diverse [4,66,67]. Different techniques related to library design have been improving continuously in the past few years. Probably the most significant change is the shift of X-ray crystallography and NMR from their traditional roles as structure providers to new roles as screening tools, thus enabling the development of fragment-based compound libraries [12,13]. Virtual screening is another tool that has improved in accuracy over the past few years, enabling smaller, target-specific libraries to be screened experimentally [18]. Even though virtual screening performs better for relatively rigid receptors regarding ligand binding, progress has also been made toward the incorporation of protein flexibility in ligand docking and scoring [15,68–75]. Computational *de novo* design is also progressing and active research to control the ability to synthesize *de-novo*-generated compound libraries is ongoing.

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